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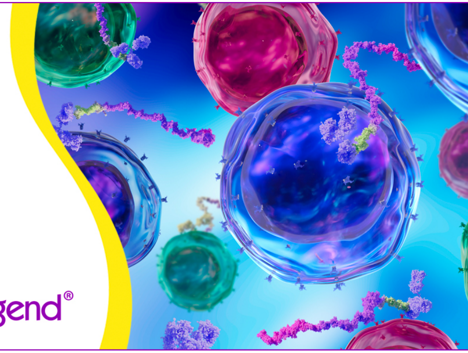

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EVIDENCE FOR INVOLVEMENT OF LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN 1 IN T CELL MIGRATION TO EPIDERMIS[1]

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Although it is well known that in various T cell-mediated skin diseases T cells migrate preferentially to epidermis, no direct evidence has been presented in which molecules on T cells are important in directing T cell traffic to epidermis. We have previously established CD4⁺ autoreactive cloned T cells with a special tropism for epidermis in vitro as well as in vivo. Antibody inhibition studies demonstrated that only anti-lymphocyte function associated Ag 1 (anti-LFA-1) mAb completely inhibited the in vitro migration of the T cells toward the epidermis, whereas mAb against other T cell surface molecules had little or no effect. Monovalent F(ab) fragment of the anti-LFA-1 mAb, although less efficient, also inhibited the T cell migration. The apparent dependency of the inhibition on the anti-α-chain mAb suggested a major role for the α-chain of LFA-1 in T cell migration to epidermis. The relevance of an LFA-1-dependent mechanism to the epidermotropic migration of T cells was further strengthened by the findings that the T cell migration to epidermis was inhibited by divalent cation depletion, cytochalasin B, and low temperature. These findings indicate that the LFA-1 molecule, which is thought to be primarily involved in cell-to-cell adhesions, also plays an important role in directing T cell migration to epidermis.

It has become increasingly clear that the epidermis is one of the most important immunologic organs, comparable with thymus. Several recent studies have suggested that the epidermis may provide a unique extrathymic microenvironment favorable for the differentiation and proliferation of certain T cells, probably through the secretion of various cytokines (1, 2). This view appears to be supported by the existence of Thy 1⁺ dendritic epidermal cells residing in the spaces between epidermal keratinocytes in mice, and of certain T cells that are postulated to "home" preferentially to the epidermis (3–5). Indeed, in various T cell-mediated skin diseases of unknown etiology such as lichen planus and lupus erythematosus, T cells migrate into the epidermis and cause the destruction of the epidermal structures. The adherence reaction between T cells and epidermal keratinocytes has been extensively studied as a possible in vitro correlate of the epidermotropic migration of T cells, and the receptor-ligand type of interaction between LFA-1[3] on T cells and LFA-1 ligand on keratinocytes has been proved to be essential for the adherence reaction (6). Despite such rapid expansion of knowledge about the molecules involved in the adherence reaction, studies of molecular mechanisms responsible for directing T cell traffic to the epidermis have been hampered by several technical difficulties. The most critical is difficulty in obtaining T cell clones with epidermotropic nature both in vivo and in vitro, and establishing relevant in vitro assays to reflect accurately their epidermotropism in vivo.

We recently have demonstrated that CD4⁺ autoreactive cloned T cells that can migrate into the epidermis upon their intradermal inoculation into the syngeneic recipients show directional migration toward the epidermis or a transformed keratinocyte cell line in an in vitro migration assay under agar gel, whereas no directional migration is seen with nonepidermotropic T cell clones (7). Our establishment of the simple quantitative in vitro migration assay has offered a unique approach for studying molecules that are essential for directing T cell migration to the epidermis.

To define the molecules involved in T cell migration to epidermis, we tested the ability of mAb against a variety of T cell surface molecules to inhibit directional movement of the T cells with a special tropism for epidermis. The in vitro T cell migration to epidermis was completely blocked by anti-LFA-1 mAb, and partially by anti-CD3 mAb, whereas mAb to other function-associated cell surface molecules had no effect. Monovalent F(ab) fragment of the anti-LFA-1 mAb also inhibited the T cell migration to the epidermis, thus excluding indirect effects mediated by the Fc portion or via cross-linking of the receptor by the divalent mAb. The involvement of LFA-1 in T cell migration to epidermis was further confirmed by the findings that the T cell migration to epidermis was dependent on the presence of divalent cations, and was susceptible to inhibition by cytochalasin B treatment and at low temperature, characteristics of LFA-1-dependent

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[3] Abbreviations used in this paper: LFA-1, lymphocyte function-associated Ag 1; MI, migration index; ICAM-1, intercellular adhesion molecule 1.

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adhesion. These findings suggest a central role for LFA-1 in directing T cell migration to the epidermis, an important first step in the complex succession of events leading to the adhesion of T cells to epidermal keratinocytes.

MATERIALS AND METHODS

Cloned T cell lines. $CD4^+$ cloned autoreactive T cells, named BB5, were derived from C57BL/6J (B6) mice and established in previous experiments (8, 9). BB5 cells, when injected into the footpads of syngeneic B6 mice, migrate into and cause the destruction of the epidermis (10, 11). Our previous study using an in vitro migration assay demonstrated that not only BB5 cells but also other cloned T cells that have been proved to be epidermotropic in vivo also showed specific directional migration to the epidermis, whereas no directional migration was seen with nonepidermotropic cloned T cells (7). The T cells were maintained by weekly feedings with mitomycin C-treated syngeneic spleen cells and rat IL-2, as described previously (8, 9).

In vitro migration assay. The technique for the in vitro migration of murine T cells has been described in detail previously (7). Briefly, a gel was prepared in a tissue culture dish (Falcon 3002) using 1% agar (Agar Noble; Difco Laboratories, Detroit, MI) in RPMI 1640 supplemented with penicillin, streptomycin, L-glutamine, and 10% heat-inactivated FCS. A row of three wells spaced 1 mm apart was cut in each agar plate using a stainless steel punch. The center well of each row received 4 μl of T cell suspension (6×10^4 cells/well). A 0.25-mm^2 fragment of epidermis prepared from footpad skin was added to the outer well as a chemoattractant. In some experiments, a transformed murine keratinocyte cell line PAM212 was added to the outer well in the place of epidermal fragments and similar results were obtained (data not shown). The inner well contained medium alone as a control. Each plate was composed of a maximum of four rows to eliminate overlap of adjacent gradients. The plates were incubated at 37°C in a fully humidified atmosphere containing 5% CO_2 in air for 48 h and was examined with an inverted microscope. The results were expressed either as absolute numbers of cells migrating to the test well or control well, or as a MI:

$$\mathrm{MI} = \frac{\text{Number of cells migrating to the test well}}{\text{Number of cells migrating to the control well}}$$

The MI shown is that to epidermis unless otherwise stated. The percent inhibition was determined by the following equation:

% Inhibition

$$= \left(\frac{\text{MI to epidermis in absence of inhibitory agents} - \text{MI to epidermis in presence of inhibitory agents}}{\text{MI to epidermis in absence of inhibitory agents} - \text{MI to medium alone}} \right) \times 100$$

This evaluation was performed in a blind fashion. Since our previous study demonstrated that the directional migration of BB5 cells to the epidermis that was expressed as an MI reached a maximal level with those harvested on day 5 after antigenic stimulation (7), those harvested on day 5 were used for all assessment of the directional migration. Because no significant directional migration of BB5 cells was observed when other tissue fragments such as syngeneic or allogeneic dermis and liver were used as attracting tissues (7), the test wells that contained medium alone were used as controls.

Antibody and other reagents. mAb used in this study is described in Table 1. The following hybridoma lines were obtained from the American Type Culture Collection (Rockville, MD): M17/4.2 (12), FD 441.8 (13), and M18/2.a.8 (13). GK1.5 (14), 145-2C11 (15), and 3JP (16) were generously provided by Dr. C. Janeway Jr. (Yale University, New Haven, CT). These mAb were purified from mouse ascites or culture supernatants by a protein A-Sepharose column. M1.70 and M17/5.2 were purchased from Hybritech Inc. (San Diego, CA). RM2.1 was raised at the Department of Immunology, Juntendo University (Tokyo, Japan) (17). Various concentrations of these mAb were added to the center well containing 6×10^4 BB5 cells at the initiation of the in vitro migration assay. Cytochalasin B was obtained from Calbiochem (San Diego, CA). One mg of cytochalasin B was dissolved in 1 ml of DMSO, then diluted 1:10 in PBS and stored at −20°C.

TABLE I
mAb used in this study

Designation	Specificity	Isotype
M17/4.2	LFA-1α (CD11a)	Rat IgG 2a
M17/5.2	LFA-1α (CD11a)	Rat IgG 2b
FD441.8	LFA-1 (CD11a)	Rat IgG 2b
M18/2.a.8	LFA-1β (CD 18)	Rat IgG 2a
M1/70	Mac-1α (CD11b)	Rat IgG 2b
GK1.5	CD4	Rat IgG 2b
145-2C11	CD3ε	Hamster IgG
RM2.1	CD2	Rat IgG
3JP	$I-A^b$	Mouse IgG 2a

F(ab) fragment preparation of anti-LFA-1 mAb. F(ab) fragments were obtained as described previously by Parham (18) with some modifications. Protein A affinity-purified M17/4.2 was adjusted to 1 mg/ml in PBS containing 0.02% sodium azide and 5 mM EDTA; 1.5 U of insoluble papain attached to carboxymethyl-cellulose beads (Sigma Chemical Co., St. Louis, MO) was added per ml of antibody solution. The reaction tube was flushed with nitrogen and rotated at 37°C for 4 h. The reaction was terminated by the removal of the papain beads by centrifugation. One-tenth volume of 0.5 M iodoacetamide in PBS was added and the mixture was dialyzed against PBS overnight at 4°C. The F(ab) fragments were purified from undigested Ig, partial digests, Fc, and $F(ab')_2$ by protein A-agarose affinity chromatography followed by fast protein liquid chromatography using a Superose 12 column (Pharmacia, Uppsala, Sweden) with the method recommended by the manufacturer. SDS-PAGE followed by silver staining of the purified F(ab) fragments revealed no detectable contamination with intact or $F(ab')_2$ antibody.

Statistical analysis. Differences between the results of experimental treatments were evaluated by means of a two-tailed Student's t test; $p < 0.05$ was considered significant.

RESULTS

Effects of mAb to T cell surface molecules on epidermotropic migration of T cells. Various cell adhesion molecules expressed on T cell surface not only are essential for cell adhesion but also play a role in T cell migration (19). To determine which T cell surface molecules are involved in the epidermotropic migration of T cells, mAb against various T cell surface molecules were tested for their ability to inhibit the in vitro migration of BB5 cells toward the epidermis. Our previous study (8) and unpublished data showed that Ag-specific function of BB5 cells such as proliferation, cytolysis, and lymphokine production is completely blocked by mAb directed against self-I-A, CD4, CD3, and LFA-1 at concentrations of 40 μg/ml. Table II shows that of mAb tested over a wide range of concentrations, only anti-LFA-1 mAb completely inhibited the in vitro migration of BB5 cells to the

TABLE II
Effects of mAb to various T cell surface molecules on the T cell migration to epidermis[a]

mAb Added, Specificity (Designation)	Concentration (μg/ml)	MI[b]	% Inhibition[c]
Expt. 1			
None		4.82 ± 1.03	—
Anti-I-A (3JP)	400	4.63 ± 1.14	5.0
	40	4.75 ± 1.60	1.8
	4	4.48 ± 0.86	8.9
Anti-CD4 (GK1.5)	400	4.42 ± 0.84	10.5
	40	4.87 ± 1.05	0
	4	4.59 ± 0.93	6.1
Anti-CD3ε (145-2C11)	400	3.17 ± 0.90	43.4 ($p < 0.05$)
	40	3.82 ± 0.86	26.3
	4	4.26 ± 0.73	14.7
Expt. 2			
None		4.52 ± 0.94	—
Anti-CD2 (RM2.1)	400	4.08 ± 0.58	12.6
	40	4.21 ± 0.90	8.9
	4	4.29 ± 0.95	6.6
Anti-LFA-1 (FD441.8)	400	1.19 ± 0.30	95.4 ($p < 0.0001$)
	40	1.23 ± 0.19	94.2 ($p < 0.0001$)
	4	1.64 ± 0.30	82.5 ($p < 0.0001$)

[a] Two representative experiments of 10 performed are shown. Similar results were obtained in each of 10 experiments.
[b] Data shown are mean MI ± SD derived from six determinations.
[c] If the percent inhibition was negative, it was considered to be zero.

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epidermis. Although partial inhibition was observed with anti-CD3 ϵ mAb at higher concentrations, the mAb was far less effective than anti-LFA-1 mAb. The in vitro migration of BB5 cells to the epidermis was not affected by three other mAb recognizing unrelated cell surface proteins: anti-CD4, anti-I-A, and anti-CD2. Thus, the specificity of inhibition observed was confirmed by the failure of the three mAb to inhibit the migration. In addition, as shown in Table III, the addition of anti-LFA-1 mAb to the center well enhanced the random migration of BB5 cells, whereas the directional migration was completely inhibited. These results argue against a nonspecific inhibitory effect of the anti-LFA-1 mAb used.

Effects of various anti-LFA-1 mAb recognizing different epitopes. LFA-1 is a heterodimer consisting of an α-chain of 180 kDa and a noncovalently associated β-chain of 95 kDa, and the β-chain is shared by LFA-1, Mac-1, and p150.95 (20). M17 reacts specifically with the α-chain, whereas M18/2.a.8 reacts with an epitope residing on the shared β-chain (12). To determine which subunits bore the epitopes involved in the epidermotropic migration of the T cells, the capacity of various mAb against LFA-1 family molecules to inhibit the epidermotropic migration was compared. As shown in Table IV, mAb against the α-chain of LFA-1, such as M17, inhibited the epidermotropic migration of BB5 cells by 86.4 to 96.5%, depending upon which mAb was used, whereas the other mAb such as anti-LFA-1β and anti-Mac-1 had no effect. This observation indicates that the α-chain of LFA-1 plays a major role in the epidermotropic migration of the T cells.

We next asked whether inhibition of the epidermotropic migration was dependent on the effects by antibody bivalency. As shown in Figure 1, the monovalent F(ab) fragment of M17/4.2 significantly inhibited the epidermotropic migration in a dose-dependent manner, although inhibition by the F(ab) fragment was partial at a concentration of 4 μg/ml, at which complete inhibition was observed with the intact IgG. Dose-dependent inhibition experiments (Fig. 1) show that the amount of the F(ab) fragment required to consistently inhibit the migration was almost 10 times higher than that of the intact IgG, consistent with a result previously reported for phorbol ester-induced leukocyte aggregation (21).

Divalent cation requirement for epidermotropic migration of T cells. Previous studies have constantly shown that LFA-1-dependent leukocyte functions such as cell-cell adhesion require divalent cations, especially Mg^{2+} (21, 22). We therefore examined whether divalent cations were required for the epidermotropic migration of the T cells. As shown in Table V, no directional migration was observed when Ca^{2+}- and Mg^{2+}-free HBSS was used as the culture medium. Addition of Ca^{2+} plus Mg^{2+} at concentrations as low as 2.5 mM restored epidermotropic migration of BB5 cells, although complete medium was more effective than HBSS added with Ca^{2+} and Mg^{2+} in supporting the epidermotropic migration. Addition of either Ca^{2+} or Mg^{2+} alone had little or no effect. Viability of BB5 cells was similar in each of the four media, but was reduced by an average of 10% as compared with controls with complete media. This pattern of divalent cation requirement has not been observed with other LFA-1 dependent leukocyte functions such as homotypic leukocyte adhesion and the binding of LFA-1$^+$ T cells to ICAM-1 in artificial membranes (21, 22).

Effects of cytochalasin B on epidermotropic migration of T cells. Since cytochalasin B has been shown to decrease lymphocyte motility via the disruption of microfilament function (23) and to inhibit LFA-1-dependent phorbol ester-stimulated aggregation of leukocytes (21), it was of interest to determine whether cytochalasin B could inhibit the epidermotropic migration. As shown in Table VI, cytochalasin B at concentrations as low as 2 μg/ml was found to completely inhibit the migration with efficiency comparable with that of anti-LFA-1 mAb. The morphologic observation under the phase microscope revealed that addition of cytochalasin B even at concentrations of 2 μg/ml significantly reduced the motile form of the T cells as compared with that observed in the absence of cytochalasin B. A loss of motile form of the T cells induced by cytochalasin B was not observed with addition of anti-LFA-1 mAb. Viability of the T cells was not affected by addition of cytochalasin B. These results suggest that epidermotropic migration of the T cells requires an intact cytoskeleton.

DISCUSSION

Our previous studies showed that epidermal invasion of the CD4$^+$ autoreactive T cells with cytolytic activity and the subsequent destruction of the epidermal structures are inhibited specifically by in vivo treatment of recipient mice with mAb against the α-chain of LFA-1

TABLE III

Addition of anti-LFA-1 mAb enhances the random migration of the T cells[a]

mAb Added, Specificity (Designation)	Concentration (μg/ml)	Attractant: Medium			Attractant: Epidermis		
		T[b]	C[c]	MI	T[d]	C[c]	MI
None		42 ± 15	41 ± 13	1.04 ± 0.15	622 ± 149	130 ± 42	5.04 ± 1.25
Anti-CD4 (GK1.5)	400	15 ± 7	16 ± 6	0.96 ± 0.12	193 ± 41	42 ± 15	4.87 ± 0.98
	40	35 ± 11	33 ± 11	1.09 ± 0.29	282 ± 74	66 ± 16	4.52 ± 1.43
Anti-CD3ϵ (145-2C11)	400	ND	ND	ND	367 ± 67	112 ± 35	3.47 ± 0.81 ($p < 0.05$)
	40	37 ± 8	36 ± 6	1.02 ± 0.16	469 ± 53	116 ± 23	4.12 ± 0.45
Anti-LFA-1 (FD441.8)	400	144 ± 32	139 ± 29	1.05 ± 0.17	226 ± 43	203 ± 50	1.13 ± 0.12 ($p < 0.0001$)
	40	131 ± 35	132 ± 22	0.99 ± 0.19	236 ± 32	182 ± 37	1.32 ± 0.18 ($p < 0.0001$)

[a] A representative experiment of five performed is shown. Similar results were obtained in each of five experiments. Data shown are mean ± SD derived from six determinations.

[b] Number of BB5 cells migrating to the test well that does not contain the epidermal fragment, but medium alone.

[c] Number of BB5 cells migrating to the control well that contains medium alone.

[d] Number of BB5 cells migrating to the test well that contains the epidermal fragment.

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TABLE IV
Inhibition of the T cell migration to epidermis by anti-LFA-1 mAb[a]

mAb Added, Specificity (Designation)	Concentration (μg/ml)	MI	% Inhibition
None		4.57 ± 1.21	—
Anti-LFA-1α (M17/4.2)	40	1.19 ± 0.20	95.5 ($p < 0.0005$)
	4	1.51 ± 0.31	86.4 ($p < 0.0005$)
	0.4	2.35 ± 0.50	62.7 ($p < 0.005$)
	0.1	3.64 ± 0.83	26.3
Anti-LFA-1α (M17/5.2)	40	1.34 ± 0.24	91.2 ($p < 0.0005$)
	4	1.49 ± 0.26	87.0 ($p < 0.0005$)
	0.4	2.68 ± 0.50	53.3 ($p < 0.01$)
	0.1	3.75 ± 0.80	23.2
Anti-LFA-1β (M18/2.a.8)	40	4.29 ± 1.37	7.9
	4	4.06 ± 0.68	14.4
Anti-Mac 1α (M1/70)	40	4.24 ± 0.63	9.3
	4	4.81 ± 0.50	0

[a] A representative experiment of three performed is shown. Similar results were obtained in each of three experiments. Data shown are mean MI derived from six determinations.

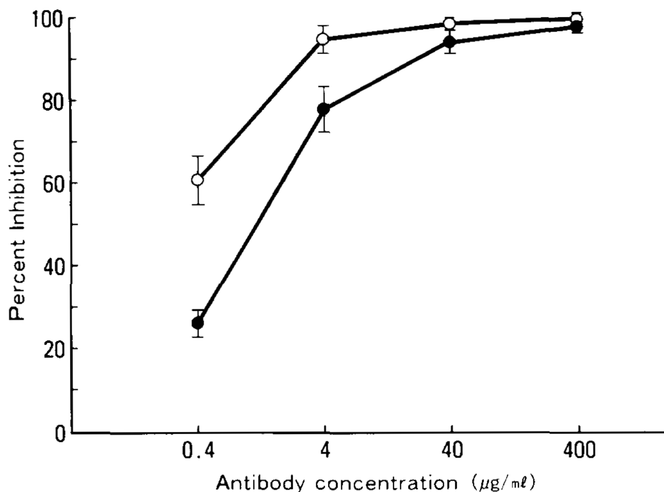

Figure 1. Dose-dependent inhibition of the T cell migration to epidermis by the intact IgG and F(ab) fragment of anti-LFA-1α mAb. Increasing concentrations of the intact IgG (○) or F(ab) fragment (●) of anti-LFA-1α mAb were added to the center well containing 6 × 10^{4} BB5 cells at the initiation of the assay. *Bar,* SD.

TABLE V
Divalent-cation dependence of the T cell migration to epidermis[a]

Divalent Cations present in Medium[b] (mM)		MI	
Mg^{2+}	Ca^{2+}	Expt. 1	Expt. 2
0	0	1.00 ± 0.13	0.98 ± 0.16
2.5	0	1.32 ± 0.31	1.62 ± 0.55
0	2.5	1.55 ± 0.34	1.46 ± 0.43
2.5	2.5	2.99 ± 0.65	3.04 ± 0.69

[a] A representative experiment of four performed is shown. Similar results were obtained each of four experiments.
[b] Before addition to the center well, BB5 cells were washed three times in Ca^{2+}- and Mg^{2+}-free PBS containing 5mM EDTA to remove divalent cations. The cells were then cultured in the center well with the incubation media indicated above: Ca^{2+}- and Mg^{2+}-free HBSS containing 10% dialyzed FCS with or without the indicated concentrations of $MgCl_2$ or $CaCl_2$. In these experiments, a gel was prepared using 1% agar in Ca^{2+}- and Mg^{2+}-free HBSS containing 10% dialyzed FCS, and the same media were used either for the inner well or for the outer well. Therefore, viability of BB5 cells in the center well was reduced by an average of 10% as compared with that in complete media (RPMI 1640) containing Ca^{2+} and Mg^{2+}.

and suggested that LFA-1 is involved in the epidermotropic migration of the T cells (24). Here we confirm and extend these findings by showing that only anti-LFA-1 mAb completely inhibited the in vitro migration of the T cells toward epidermis, whereas mAb against other T cell surface molecules expressed at comparable or higher densities had little or no effect, although these mAb appeared to equally block Ag-specific functions of the T cells in vitro and in vivo, such as proliferation, cytolysis, and delayed-type hypersensitivity responses. The observation that the epidermotropic migration was completely blocked by mAb against LFA-1 indicates that LFA-1 molecule on the T cells plays an important role in directing the T cell migration to epidermis. The finding that the anti-LFA-1 mAb rather enhanced random migration of BB5 cells makes it unlikely that observed inhibitory effect of anti-LFA-1 mAb on the epidermotropic migration may be secondary to the decreased adhesion of the T cells to the substratum. Our recent observation that LFA-1 molecule is preferentially expressed at high densities on the surface of epidermotropic T cell clones including BB5 cells whereas nonepidermotropic T cell clones are very weakly positive (25) supports this contention. The relevance of an LFA-1-dependent mechanism to the epidermotropic migration of T cells is further strengthened by the findings in this study that the T cell migration to epidermis was dependent on the presence of divalent cations (Table V) and was susceptible to inhibition by cytochalasin B treatment (Table VI) and low temperature (4°C) (data not shown), all of which have been shown previously to be characteristics of LFA-1-dependent adhesion (21, 22).

TABLE VI
Effect of cytochalasin B on the T cell migration to epidermis[a]

Treatment	MI	% Inhibition
Expt. 1		
None	4.33 ± 1.06	—
DMSO[b]	4.11 ± 1.05	6.6
Cytochalasin B (10 μg/ml) in DMSO[c]	1.70 ± 0.34	78.5 ($p < 0.0005$)
Cytochalasin B (2 μg/ml) in DMSO[c]	1.96 ± 0.71	70.7 ($p < 0.005$)
Expt. 2		
None	4.84 ± 1.36	—
DMSO[b]	5.01 ± 0.57	0
Cytochalasin B (20 μg/ml) in DMSO[c]	1.31 ± 0.33	93.1 ($p < 0.0005$)
Cytochalasin B (2 μg/ml) in DMSO[c]	1.81 ± 0.26	80.0 ($p < 0.001$)

[a] Two representative experiments of six performed are shown. Similar results were obtained in each of six experiments.
[b] As controls, DMSO was added to the center well at the concentration of 1% v/v, which corresponded to the amount of DMSO present in cultures with 10 μg/ml of cytochalasin B.
[c] Cytochalasin B dissolved in DMSO was added to the center well containing 6 × 10^{4} BB5 cells at the initiation of the assay at the indicated concentration.

However, also included in our results were several observations that seem to contradict LFA-1-dependent adhesion previously reported by others (21, 22). First, addition of neither Ca^{2+} nor Mg^{2+} alone restored the T cell migration to epidermis, whereas in other LFA-1-dependent adhesion systems Mg^{2+} alone, but not Ca^{2+} alone, produced binding equivalent to that which occurred in the presence of both divalent cations (21, 22). In the interaction of LFA-1 with ICAM-1, at least two divalent cation binding sites have been postulated: a high affinity site specific for Mg^{2+} and a low affinity site that binds either Mg^{2+} or Ca^{2+} (26). In contrast, a new gene family termed "selections" of adhesion proteins, such as lymph node homing receptor, has also been proposed to have at least two divalent cation binding sites, a high-affinity site requiring Ca^{2+} and a low-affinity site requiring either

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Mg^{2+} or Ca^{2+} (27). Therefore, the requirement for both divalent cations to support the migration may indicate that both bind separate binding sites and suggests that an LFA-1/ICAM-1-independent mechanism may also be involved in the T cell migration to epidermis demonstrated here; for instance, the interactions of other adhesion molecules such as "selectins" with their ligands.

Second, an apparent dependence of the T cell migration to epidermis on the α-chain of LFA-1 was again never observed in previous reports on LFA-1-dependent cell adhesion, in which both the anti-α- and anti-β-chain mAb are equally effective (21, 22). This finding appears to be in contrast to previous studies by Van Epps et al. (28). They have demonstrated a major role for the β-chain in T cell migration: anti-β-chain mAb was much more effective than anti-α-chain mAb at inhibiting the in vitro migration of human lymphocytes to lymphocyte chemotactic factor, whereas the IL-2-mediated migration was marginally inhibited by either anti-α-chain mAb or anti-β-chain mAb. Because the cellular system they employed was a heterogeneous population of human peripheral blood lymphocytes, in which great variation could exist in the rate of migration, this difference may simply reflect the difference in mechanisms for cell migration between murine cloned T cell lines with high migratory capacity in this study and human heterogeneous resting populations used for their study. An alternative and most likely interpretation given the result is that the anti-β-chain mAb used in this study probably does not recognize epitopes on LFA-1 relevant to T cell migration to epidermis: binding of the anti-α-chain mAb, but not of the anti-β-chain mAb, could interfere with the interaction of LFA-1 with the ligand derived from epidermis that could serve to direct T cell migration to epidermis.

The possibility remains to be excluded that the observed inhibition of the in vitro epidermotropic migration by the anti-LFA-1 mAb was secondary to binding of the mAb to the epidermal fragments in the outer well, thereby preventing a ligand for LFA-1 from diffusing through the agar. The epidermal fragments therefore were stained with anti-rat IgG alone to determine the presence and distribution of the mAb added to the center well by a direct immunofluorescent technique. No in vitro binding of the mAb to the epidermal fragments was observed (T. Shiohara, unpublished data). These results argue strongly against any possibility that the in vitro binding of the anti-LFA-1 mAb to the epidermal fragments may be responsible for the observed effects of the mAb on epidermotropic migration of the T cells.

Although our data indicate that LFA-1 is involved in T cell migration to epidermis, at present comprehensive explanations of how the LFA-1 molecule can participate in T cell migration to epidermis are unavailable. Several possible explanations, however, can be offered. One possible explanation is that LFA-1 might be itself a receptor that receives the necessary signal from the epidermis for directing the T cell migration to the epidermis. Whether ICAM-1 could also function as the ligand for LFA-1 in this process as observed in the LFA-1-dependent adhesion is not yet clear. Considering the fact that the epidermotropic T cells can respond to keratinocyte-derived cytokines such as granulocyte-macrophage colony-stimulating factor (T. Shiohara, unpublished data), it is possible that these keratinocyte-derived cytokines could be additional candidates for the LFA-1 ligand in this process. Whether these cytokines or ICAM-1 functions as the ligand for LFA-1 in T cell migration, LFA-1 on the T cells would be ligated preferentially in the area of the cell surface facing the epidermis. Such localized receptor ligation would provide a mechanism for directing cell movement to the epidermis capable of releasing these cytokines and ICAM-1. In this regard, studies to determine whether treatment of the epidermis with inhibitors of protein synthesis could affect the ability to induce the T cell migration to epidermis may be worth exploring. A second possibility is that binding of anti-LFA-1 mAb to LFA-1 on the T cells would deliver a negative signal to the T cells, thereby rendering them unresponsive to the ligand.

The integrin receptors have been shown to have an affinity for talin, a cytoskeletal protein associated with the actin filament network (29). Thus, the ligand binding to LFA-1 may induce changes in the distribution or conformation of such cytoskeletal proteins, thereby providing contractile force for directional movement. In this regard, it has been demonstrated that the clustering of LFA-1 on the surface of the T cell, where it is in contact with the Ag-presenting B cell, is accompanied by the accumulation of the cytoskeletal protein under the membrane of the T cell in the cell contact region (30). Based on the findings, it is reasonable to speculate that the localization of the LFA-1 molecule to the area of the cell membrane facing toward the epidermis may induce polarization of cytoskeletons in the direction of subsequent cell migration, which could serve to direct the cell migration to the epidermis. Our preliminary experiments, however, showed that the clustering of LFA-1 never occurred on the migrating T cells, and LFA-1 had no obvious membrane localization to the leading edge or the uropod area of the T cells: no change in cell-surface density of LFA-1 was observed between the leading edge of the T cells facing the epidermis and the trailing edge (T. Shiohara, et al., unpublished data). Thus, redistribution of cytoskeletal proteins associated with LFA-1 to the leading edge of the T cells is unlikely to account for the T cell migration to epidermis.

An alternative explanation for LFA-1-dependent migration mechanism(s) would be that a conformational change, but not changes in the density, that is induced in the LFA-1 molecule may alter the affinity for its ligand and provide a mechanism for directional movement of the T cells. Because Dustin and Springer (31) demonstrated that TCR ligation leads to energy-dependent conversion of LFA-1 to a high-avidity state, differences in the avidity of adhesion proteins between the leading and trailing edges of the T cells could induce directed migration of the T cells. Thus, elucidation of the LFA-1-dependent migration mechanism(s) will allow greater understanding of how T cells migrate to epidermis and shed further light on the exact role of LFA-1 in vivo.

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